

We claim:

1 1. A method for measuring protein S activity in a plasma sample, comprising the
2 steps of:

3 (a) mixing a sample of test plasma with PS-deficient plasma, tissue factor (TF),
4 phospholipid (PL), calcium and activated protein C (APC) and measuring the clotting
5 time of the sample; and

6 (b) comparing the measurement in (a) to a standard curve derived from the
7 clotting time of plasma samples having a range of known protein S activities.

1 2. A method for measuring protein S activity in a plasma sample, comprising the
2 steps of:

3 (a) preparing a standard curve by mixing plasma samples having a range of
4 protein S activities with PS-deficient plasma, tissue factor (TF), phospholipid (PL) and
5 activated protein C (APC), measuring the clotting time and plotting clotting time vs.
6 protein S activity;

7 (b) mixing a sample of test plasma with PS-deficient plasma, TF, PL, calcium and
8 APC and measuring the clotting time of the plasma sample; and

9 (c) comparing the measurement in (b) to the standard curve prepared in (a).

1 3. A method for measuring protein S activity in a plasma sample, comprising the
2 steps of:

3 (a) mixing a sample of test plasma with PS-deficient plasma, tissue factor (TF),
4 phospholipid (PL), calcium and an activator of Protein C (PCA) and measuring the
5 clotting time of the sample; and

6 (b) comparing the measurement in (a) to a standard curve derived from the
7 clotting time of plasma samples having a range of known protein S activities.

1 4. A method for measuring protein S activity in a plasma sample, comprising the
2 steps of:

3 (a) preparing a standard curve by mixing plasma samples having a range of
4 protein S activities with PS-deficient plasma, tissue factor (TF), phospholipid (PL),
5 calcium and an activator for Protein S (PCA), measuring clotting time, and plotting
6 clotting time vs. protein S activity;

7 (b) mixing a sample of test plasma with PS-deficient plasma, TF, PL and a PCA
8 and measuring the clotting time of the plasma sample; and

9 (c) comparing the measurement in (b) to the standard curve prepared in (a).

1 5. The method of any one of claims 1-4, wherein the TF is recombinant.

1 6. The method of any one of claims 1-4, wherein the recombinant TF is rabbit TF.

1 7 The method of any one of claims 1-4, wherein the TF is purified from mammalian
2 cells.

1 8. The method of any one of claims 1-4, wherein the PL is synthetic.

1 9. The method of any one of claims 1-4, wherein the PL comprises 1,2-dioleoyl-sn-
2 glycerol-3-phosphocholine (PC), 1,2-dioleoyl-sn-glycerol-3-phospho-L-serine (PS), and
3 1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine (PE).

1 10. The method of any one of claims 1-4, wherein the molar ratio of PC:PS:PE is
2 about 3 to about 4 to about 5.

1 11. The method of claim 1 or 2, wherein the activated protein C was activated prior to
2 the assay by thrombin.

1 12. The method of claim 1 or 2, wherein the activated protein C was activated prior to
2 the assay by snake venom.

- 1 13. The method of claim 1 or 2, wherein the activated protein C was derived from
2 recombinant Protein C.
- 1 14. The method of claim 1 or 2, wherein one or more of the PS-deficient plasma, TF
2 and APC are derived from a mammalian source selected from the group consisting of a
3 cow, a pig, and a rabbit.
- 1 15. The method of any one of claims 1-4, wherein one or more of the PS-deficient
2 plasma, TF and APC are derived from a human.
- 1 16. The method of any one of claims 1-4, wherein the variation of calibration curves
2 has a <3% coefficient of variation (CV) over a period of 2 hours.
- 1 17. The method of any one of claims 1-4, wherein the variation of calibration curves
2 has a <3% coefficient variation (CV) over a period of 8 hours.
- 1 18. The method of any one of claims 1-4, wherein the variation of calibration curves
2 has a <3% coefficient variation (CV) over a period of 2 weeks.
- 1 19. The method of any one of claims 1-4, wherein the assay has a <3% within-run
2 coefficient of variation (CV).
- 1 20. The method of any one of claims 1-4, whereas the measuring step is chromogenic.
- 2 21. The method of any one of claims 1-4, whereas the measuring step is
3 spectrophotometric.
- 1 22. The method of claim 1 or 3, further comprising the step of measuring the clotting
2 time of a normal control plasma sample with known protein S activity and comparing that
3 clotting time to the clotting time in step (a) or step (b).
- 1 23. A kit for measuring the functional activity of protein S (PS) in a plasma sample,
2 said kit comprising one or more containers containing PS-deficient plasma, tissue factor
3 (TF), phospholipid (PL), calcium and/or activated Protein C (APC).

1 24. A kit for measuring the functional activity of protein S (PS) in a plasma sample,
2 said kit comprising one or more containers containing PS-deficient plasma, tissue factor
3 (TF), phospholipid (PL), calcium and/or protein C activator (PCA).

1 28. The kit of claim 23 or 24, further comprising calibration plasma comprising about
2 100% protein S for preparing a standard curve.

1 25. The kit of claim 23 or 24, further comprising normal control plasma comprising
2 between about 40-50% protein S.